define the problems which must be solved, (2) decide what needs to be done and (3) develop a strategy of collaboration and coordination to get it done. This is long overdue and should now be accomplished as soon as possible, before any sort of nationalized health care juggernaut begins to gather momentum in the nation. Actually there may be very little time to spare. The scenario may have already started.

—MSMW

Phenylketonuria and Its Variants

OF ALL THE 150 or 200 inborn errors of metabolism known today, phenylketonuria (PKU) is the best known and the most studied. The reasons for this are clear: PKU is one of the commonest of these diseases (one in about 14,000 live births in the United States, even commoner in some ethnic groups), if untreated it leads to lifelong severe mental retardation (more devastating to the average family than, say, death in infancy) and an effective treatment is readily available. It is this last feature that has led, over the last 30 years, to the great interest in PKU among pediatricians and has caused many states to introduce screening of all neonates, as described by Dr. Charles Parker elsewhere in this issue. We can control the clinical features of PKU better than those of most inborn errors of metabolism—it is paradoxical though rewarding that our very success in treating PKU will in future severely limit opportunities to study the natural history of the disease. On the purely scientific side there have also been advances, though these have been slower than in clinical management and screening.

The collaborative study of children treated for PKU, referred to by Dr. Parker, was originally set up to determine whether dietary treatment had any value. Although at the time some felt this to be supererogatory, much valuable information has been produced by the study and our knowledge of PKU has been materially advanced by it. In the last few years, the collaborative study has been concentrating on when the dietary treatment should be terminated. Even in the early 1950's, pediatricians hoped that a normal diet could be resumed after a few years and some were courageous enough (or were forced by circumstances) to try the experiment. Scattered conflicting reports of the effects of terminating treatment appeared in the literature and the collaborative study has started a systematic investigation.

The low-phenylalanine diet was introduced because it was hypothesized that the high concentration of phenylalanine in the blood damaged the brain from soon after birth onwards and, as often happens with brain damage in infancy, this showed itself as global mental retardation.[1] However, a similar insult to a more mature brain would be expected to produce a different spectrum of signs and symptoms; although there might well be intellectual deterioration—that is, dementia—this would probably be relatively slow, and a more sensitive indicator of late onset phenylalanine intoxication would be desirable. Intelligence quotient tests were first used in the early 1950's for following effects of dietary treatment[1] and have been widely used since. They are probably the best indicators we have of brain damage caused by hyperphenylalaninemia in early infancy; however, there are better tests for the brain damage that might result from late onset phenylalanine intoxication—for example, tests of attention span, the categories subtest of the Reitan-Halstead battery, and structured or semistructured psychiatric interviews.

The early literature reported a handful of cases of "atypical PKU": cases of persons with normal or near normal intelligence quotients and with substantially raised blood phenylalanine levels and urinary excretion of phenylpyruvic acid and other "abnormal" phenylalanine metabolites. With improved laboratory techniques it was shown that these persons had blood phenylalanine concentrations well below those of typical patients with PKU, though considerably above the normal. The frequency with which these cases occurred became apparent when screening of the newborn was introduced—about one in 30,000 live births or one in three of all those with hyperphenylalaninemia in the United States were affected. These cases were labeled hyperphenylalaninemia variants or *hyper-phe*. Phenylalanine hydroxylase is the enzyme that normally converts phenylalanine to tyrosine but is absent or inactive in persons with typical PKU; in those with variant forms of hyperphenylalaninemia there is appreciable phenylalanine hydroxylase activity in the liver, though far less than in the normal. In some of these persons there is evidence suggesting a qualitatively altered phenylalanine hydroxylase which in turn suggests a structural gene mutation,

in others there is insufficient evidence to decide whether a structural gene or a regulator gene has undergone mutation. Most patients with variant forms of hyperphenylalaninemia do not require dietary treatment after the first few months of life, although the decision whether to treat, and for how long, requires both careful investigation and clinical judgment. Laboratory findings range from the almost normal to those of typical PKU with no discontinuities and, as far as we know, clinical effects parallel the blood phenylalanine concentrations. As Dr. Parker points out, whether one classifies a child as having PKU or as *hyperphe* depends on an arbitrary set of criteria and near the borderline should not involve any difference in treatment.

Studies of phenylalanine hydroxylase have advanced at a snail's pace since the identification of this enzyme by Mitoma and colleagues[2] and by Wallace and co-workers[3] in 1957. Only in the last five or six years have a number of laboratories in different countries taken up the problem and progress has accelerated. Two groups, Danks, Cotton and associates[4,5] in Melbourne, Australia, and Bessman, Barranger, Parker and co-workers in Los Angeles[6,7] have used innovative approaches in which the crude liver homogenate supernatant is subjected to a single chromatographic separation without the laborious preliminary protein separations usually involved in isolating enzymes. The Australian group uses affinity chromatography; that is, the ability of phenylalanine hydroxylase to bind specifically to its cofactor which has been immobilized by binding to sepharose. The Los Angeles group uses columns of calcium phosphate gel. Both methods have the advantages of speed and simplicity—many samples can be investigated in the time it takes to make a single preparation by conventional methods. However, there are difficulties in the interpretation of the results obtained using these novel techniques and, in both cases, the phenylalanine hydroxylase preparations need to be compared with the enzyme prepared by traditional methods, which has not yet been done. Nevertheless, affinity chromatography is a powerful technique and it seems likely that much of our information on phenylalanine hydroxylase over the next few years will come from the Australian group.

Several workers have reported that phenylalanine hydroxylase, as isolated, is polydisperse, at least two forms of different molecular weights or isoelectric point (net charge) or both being present. This is, at present, the area of greatest obscurity. One group[8] found that filtration through glass wool, a technique that splits certain complexes, converted the higher molecular weight forms to the lower molecular weight (about 108,000) form also present. Many workers are approaching the conclusion that the relevant gene codes for a polypeptide of molecular weight about 54,000, that a dimer of this polypeptide is enzymically active and that higher molecular weight and other forms of the enzyme are either the results of posttranslational changes or artifacts arising during isolation. Phosphorylated forms of the enzyme have been described, though it is not clear that these play any part in regulating enzymic activity *in vivo*. Phosphorylated enzymes with their relevant kinases and phosphatases generally are associated with metabolism of, for example, glycogen (where the body may be subjected to sudden loads or demands and rapid regulation of enzymic activity is necessary) but there is no evidence that phenylalanine hydroxylase falls into this category.

The three forms of phenylalanine hydroxylase, pi, kappa and upsilon, described by Dr. Parker probably represent posttranslational changes in the phenylalanine hydroxylase molecule resulting in an alteration of net charge. Because the molecular weights are reported[7] to be in the range 135,000 to 150,000, the dimer molecule must be complexed with some molecule or molecules of total weight 27,000 to 42,000. The term *isozyme* should not be applied to such posttranslational complexes; conventionally *isozymes* or *isoenzymes* are taken to be two or more different protein molecules with similar enzymic activity coded for by two or more nonallelic genes, and usually occurring in different tissues—an example is the pyruvate kinases of erythrocytes and leucocytes. The term isozyme is not applied to the different enzymes produced by various mutations of a single structural gene (the single locus multiple allele case). Because PKU and the variant forms of hyperphenylalaninemia are genetically determined—that is, are the results of gene mutation—it is difficult to see how variations in the relative amounts of the three posttranslational complexes, pi, kappa and upsilon, can be related to different degrees of severity of the disease. The suggestion that we are dealing with multiple loci rather than multiple alleles[7] runs counter to all the genetic evidence. It could be postulated that a structural gene mutation might lead to a protein with a re-

duced tendency to form certain complexes, but there is no evidence on this point and, in any case, its possible relevance to any clinical features is obscure. Possibly more relevant is recent evidence that formation of these complexes is under hormonal control.[9]

Dr. Bessman has long been critical of the dietary treatment of PKU and of the intoxication hypothesis on which the treatment is based. He has put forward an alternative, the justification hypothesis.[10] This postulates that, because heterozygotes for PKU have only half the normal amount of phenylalanine hydroxylase, a pregnant heterozygote will supply less than the optimum amount of tyrosine to all her offspring *in utero* and, should a fetus be homozygous for the PKU gene, it will be unable to convert phenylalanine to tyrosine and will have severe tyrosine deficiency. According to the justification hypothesis the severe or profound mental retardation of PKU results from this tyrosine deficiency *in utero*. There is, however, strong evidence that such fetuses do not have tyrosine deprivation *in utero* which, as far as PKU is concerned, disproves the justification hypothesis. Many hundreds of infants with PKU have by now been treated with the low-phenylalanine diet and are mentally normal. This would not be expected if the mental retardation of untreated PKU were the result of events *in utero*. Fasting plasma tyrosine concentrations in heterozygotes (1.042 ± 0.23 mg per 100 ml, $n = 34$) were not greatly different from those in normal controls (1.127 ± 0.19 mg per 100 ml, $n = 39$).[11] There is little information on blood tyrosine levels during pregnancy or on variations during the course of the day.

Tyrosine is an essential amino acid for those with PKU—before as well as after birth. Hence tyrosine deficiency *in utero,* like protein deficiency, would result in a low birth weight in relation to gestational age. The four studies of birth weights of infants born to mothers who are obligate heterozygotes for PKU all showed no differences between these infants and normal controls,[12-15] moreover there were no differences between the birth weights of infants with PKU and of their unaffected siblings of whom presumably two thirds were heterozygotes and a third homozygous normal. What evidence there is suggests that, if partial deprivation of protein or an essential amino acid occurs in fetal life, the brain is to some extent spared relative to other organs; there is no reason to expect that tyrosine deficiency would affect the brain but leave the body weight unaffected and, in fact, the head circumferences of infants with PKU are normal at birth.[15] In older untreated patients with PKU there is pronounced microcephaly, which must arise postnatally. However, those treated with a low-phenylalanine diet since earliest infancy have normal head circumferences. Hence, the justification hypothesis should be rejected for PKU. There is no evidence for tyrosine deficiency *in utero* or that this causes mental retardation either in homozygotes or heterozygotes. The justification hypothesis, however, may be correct for some other aminoacidopathies. Each condition will have to be investigated separately.

LOUIS I. WOOLF, PhD
Kinsmen Laboratory of Neurological Research
Department of Psychiatry
University of British Columbia
Vancouver, BC
Canada

REFERENCES

1. Woolf LI, Griffiths R, Moncrieff AA: Treatment of phenylketonuria with a diet low in phenylalanine. Brit Med J 1:57-64, 1955

2. Mitoma C, Auld RM, Udenfriend S: On the nature of the enzymatic defect in phenylpyruvic oligophrenia. Proc Soc Exp Biol Med 94:634-635, 1957

3. Wallace HW, Moldave K, Meister A: Studies on the conversion of phenylalanine to tyrosine in phenylpyruvic oligophrenia. Proc Soc Exp Biol Med 94:632-633, 1957

4. Cotton RGH, Danks DM: Purification of inactive phenylalanine hydroxylase protein from liver in classical phenylketonuria. Nature (Lond) 260:63-64, 1976

5. Cotton RGH, Grattan PJ: Phenylalanine hydroxylase of Macaca irus, a simple purification by affinity chromatography. Eur J Biochem 60:427-430, 1975

6. Barranger JA, Geiger PS, Huzino A, et al: Isoenzymes of phenylalanine hydroxylase. Science 175:903-905, 1972

7. Barranger JA: The implications of multiple forms of phenylalanine hydroxylase in phenylketonuria and related diseases of phenylalanine metabolism. Biochem Med 15:55-86, 1976

8. Woo SLC, Gillam SS, Woolf LI: The isolation and properties of phenylalanine hydroxylase from human liver. Biochem J 139:741-749, 1974

9. Miller MR, Shiman R: Hydrocortisone induction of phenylalanine hydroxylase isozymes in cultured hepatoma cells. Biochem Biophys Res Comm 68:740-745, 1976

10. Bessman SP: Genetic failure of fetal amino acid "justification": A common basis for many forms of metabolic, nutritional and "non-specific" mental retardation. J Pediat 81:834-842, 1972

11. Woolf LI, Cranston WI, Goodwin BL: Genetics of phenylketonuria. Nature (Lond) 213:882-883, 1967

12. Woolf LI: A study of the cause of the high incidence of phenylketonuria in Ireland and West Scotland. J Ir Med Assoc 69:398-401, 1976

13. Rothman KJ, Pueschel SN: Birthweight of children with phenylketonuria. Pediatrics 58:842-844, 1976

14. Smith I, Carter CO, Wolff OH: Heterozygote advantage for the phenylketonuria allele. J Med Genet 15:246-248, 1978

15. Holm VA, Kronmal RA, Williamson M, et al: Physical growth in phenylketonuria: II. growth of treated children in the PKU collaborative study from birth to 4 years of age. Pediatrics 63:700-707, 1979